Amendments to the Claims

Please cancel claims 22, 23 and 24.

1. (Original) A method of evaluating a compound for utility in treating neurological disease comprising contacting a compound with a cell that coexpresses KCNQ2 and KCNQ3, wherein the KCNQ2 and the KCNQ3 form a potassium channel; and measuring the activity of the potassium channel.

2. (Original) The method of Claim 1 wherein the cell is an oocyte.

3. (Original) The method of Claim 1 wherein the cell is a mammalian cell.

4. (Original) The method of Claim 1 wherein the cell is a mammalian cell selected from HEK 293E, CHO and COS cells.

5. (Original) The method of Claim 1 wherein KCNQ2 is hKCNQ2.

6. (Original) The method of Claim 1 wherein KCNQ3 is hKCNQ3.

7. (Original) The method of Claim 1 wherein the compound is an agonist of the potassium current.

8. (Original) The method of Claim 1 wherein the compound is an antagonist of the potassium current.

9. (Original) The method of Claim 1 wherein the activity of the potassium channel is measured by a current or a change in membrane voltage, wherein the change in membrane voltage is determined through a voltage sensitive dye.

10. (Currently amended) The method of claim 9 wherein the voltage sensitive dye is detectable by fluorescence ~~fluoresence~~.

11. (Original) The method of Claim 1 comprising contacting a compound with a mammalian cell that coexpresses KCNQ2 and KCNQ3, wherein the KCNQ2 and the KCNQ3 form a potassium channel; and measuring the activity of the potassium channel.

12. (Original) The method of Claim 11 wherein the compound is an agonist of the potassium current.

13. (Original) The method of Claim 11 wherein the compound is an antagonist of the potassium current.

14. (Original) The method of Claim 11 wherein the activity of the potassium channel is measured by a current.

15. (Original) The method of Claim 11 wherein the activity of the potassium channel is measured by a change in membrane voltage wherein the change in membrane voltage is determined through a voltage sensitive dye.

16. (Currently amended) The method of claim 15 wherein the voltage sensitive dye is detectable by fluorescence ~~fluoresence~~.

17. (Original) The method of Claim 1 comprising contacting a compound with a mammalian cell that coexpresses hKCNQ2 and hKCNQ3, wherein the hKCNQ2 and the hKCNQ3 form a potassium channel; and measuring the activity of the potassium channel.

18. (Original) The method of Claim 17 wherein the compound is an agonist of the potassium current.

19. (Original) The method of Claim 17 wherein the compound is an antagonist of the potassium current.

20. (Original) The method of Claim 17 wherein the activity of the potassium channel is measured by a current or a change in membrane voltage wherein the change in membrane voltage is determined through a voltage sensitive dye.

21. (Currently amended) The method of claim 20 wherein the voltage sensitive dye is detectable by fluorescence ~~fluoresence~~.

22-24. (Canceled)

Amendments to the Specification

Please amend the Specification as follows:

On page 1 immediately below the Title please insert the following paragraph:

Cross Reference To Related Applications

This application is a Divisional of Serial Number 09/454,868 filed on December 3, 1999 and claims priority to, U.S. Provisional Patent Application Serial Number 60/110,804, filed December 3, 1998, herein incorporated by reference in its entirety.

Please amend the paragraph beginning on page 3, line 12 and continuing through line 17 as follows:

An object of present invention is to use KCNQ2 and KCNQ3 gene coexpression to screen for pharmacologically active compounds in high throughput ~~thoughput~~ screening assays. The development of high throughput screening assays has tremendous commercial utility in the discovery of compounds useful in the treatment of neurological disorders.

On page 4, please delete the paragraph beginning on line 7 and ending on line 24 and replace it with the following rewritten "Brief Description of the Drawings":

Brief Description of the Drawings

Figure 1A is a series of traces depicting currents recorded in *Xenopus* oocytes following injection of KCNQ2 mRNA, KCNQ3 mRNA or an equimolar ratio of KCNQ2 and KCNQ3 mRNAs.

Figure 1B is a histogram showing the average current response to a voltage-clamp step to 0 mV from -70 mV in cells injected with KCNQ2, KCNQ3 or an equimolar ratio of KCNQ2 and KCNQ3 mRNAs (45 ng of each mRNA was injected per oocyte).

Figure 1C is a series of traces depicting the effect of 1 mM TEA on currents elicited from oocytes injected with KCNQ2 mRNA or an equimolar ratio of KCNQ2 and KCNQ3 mRNAs.

Figure 1D is a plot depicting dose response curves for TEA block of KCNQ2 channels and KCNQ2+KCNQ3 channels.

Figure 2A is a series of traces depicting current response to traditional M-current voltage clamp protocol for native current and KCNQ2+KCNQ3 channels.

Figure 2B is a series of traces depicting activation of M-current and KCNQ2+KCNQ3 channels from a holding potential of -60 mV in 5 mV increments.

Figure 2C is a series of plots depicting conductance-voltage curves fitted with a single Boltzmann function.

Figure 2D is a series of traces depicting the observation that the deactivation process had two time constants for both channel types.

Figure 2E is a series of plots depicting the reciprocal time constant for fast deactivation of the native M-current and KCNQ2+KCNQ3 channels.

Figure 3A is a series of traces depicting the blockade of M-current in SCG neurons by XE991.

Figure 3B is a series of traces depicting the blockade of KCNQ2+KCNQ3 channels by XE991.

Figure 3C is a series of plots depicting Dose-response curves for linopirdine (open symbols) and XE991 (closed symbols) for blockade of M-current.

Figure 3D is a series of plots depicting Dose-response curves for linopirdine (open symbols) and XE991 (closed symbols) for blockade of KCNQ2+KCNQ3 channels.

Figure 3E is a series of traces depicting the effect of 10 μM XE991 on the firing properties of phasic sympathetic neuron recorded from the SCG.

Figure 4A is a histogram showing the distribution of phasic neurons in prevertebral and paravertebral sympathetic ganglia.

Figure 4B is a photograph of a gel depicting KCNQ2 mRNA expression in sympathetic ganglia.

Figure 4C is a photograph of a gel depicting KCNQ3 mRNA expression in sympathetic ganglia.

Figure 4D is a photograph of a gel depicting KCNQ2 mRNA expression in three brain regions.

Figure 4E is a photograph of a gel depicting KCNQ3 mRNA expression in three brain regions.

Figure 5 is a plot depicting the effect of 0.3 μM XE991 on hKCNQ2 expressed in a stable line of HEK-293E cells.

Figure 6 is a plot depicting the observation that linopridine induces a time- and concentration-dependent increase in fluorescence of HEK 293E cells stably expressing the hKCNQ2 potassium channel.

Figure 7 is a plot depicting the relative effects of several M-current modulators on the fluorescence of HEK 293E cells stably expressing the hKCNQ2 potassium channel.

Please amend the paragraph beginning on page 5, line 13 and continuing through line 17 as follows:

It is also preferred that the activity of the potassium channel is measured by a current or a change in membrane voltage through a voltage sensitive dye wherein it is more preferred that the voltage sensitive dye is detectable by fluorescence ~~fluoresence~~.

Please amend the paragraph beginning on page 7, line 6 and continuing through page 8, line 9 as follows:

As used herein, a method of evaluating a compound for utility in treating neurological disease can be preformed in a number of ways. Any suitable container for contacting a compound to be evaluated with a cell in which the KCNQ2 and KCNQ3 gene has been expressed is envisaged. Such containers can be single or multiple. A preferred example is a well in a plate, preferably ~~preferrably~~ a multiwell plate; especially a multiwell plate designed for high throughput ~~troughput~~ screening assays. The plate typically has 96 or 384 wells, but may have more, up to the limits of measuring activity in the well.

As used herein, a cell that coexpresses KCNQ2 and KCNQ3, is meant to mean any cell, mammalian or non-mammalian ~~nonmammalian~~, wherein the KCNQ2 and the KCNQ3 can be expressed to form a voltage-gated ~~volatge-gated~~ potassium channel. All forms of KCNQ2 and KCNQ3 are envisaged which will correlate to the M-channel current. Equivalent forms of KCNQ2 and KCNQ3 may include non-mammalian forms ~~nonmammalian~~, such as from Drosophila ~~Drosiphila~~ or C. Elegans, or may be mammalian, such as forms from primate, rat or human. Human and rat are preferred, human is most preferred. It is preferred that the cell is an

oocyte or a mammalian cell. It is more preferred that the mammalian cell be a HEK 293E, a CHO or a COS cell.

As used herein, measuring the activity of the potassium channel can be performed in a number of ways. One method of "measuring the activity" is to measure the current of the potassium channel, such methods are well known in the art. A second method envisaged for "measuring the activity" is through the use of a change in membrane voltage. Changes in membrane voltage can be determined by use of a voltage sensitive dye wherein it is preferred that the voltage sensitive dye is detectable by fluorescence ~~fluoresence~~. Example of a voltage sensitive dye is dibac. A third method for "measuring the activity" is ^{86}Rubidium efflux assay.

Please amend the paragraph beginning on page 13, line 15 and continuing through line 32 as follows:

Method: Full-length KCNQ2 cDNAs were amplified from adult human brain cDNA using the ~~following~~ primers ~~(~~CCCCGCTGAGCCTGAG (SEQ ID NO: 1), TGTAAAAGGTCACTGCCAGG (SEQ ID NO: 2)~~)~~ with the Expand Fidelity enzyme mixture (Boehringer Mannheim, Indianapolis). The KCNQ2 cDNA clone used in the biophysical studies was identical to the KCNQ2 cDNA isolated previously from a fetal brain cDNA library (Singh et al., 1998) except that it had a small deletion in the carboxy intracellular domain (30 amino acids from residues 417 to 446). This region is also alternatively spliced in the KCNQ2 cDNA clone described by Biervert et al. (1998). Preparation, injection of cRNA and recording from oocytes was performed at room temperature as described previously (Dixon et al. 1996). The standard extracellular recording solution contained: 82 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM Na-HEPES (pH 7.6). Data collection and analysis were performed using pClamp software (Axon Instruments, Foster City, CA).

Please amend the section beginning on page 18, line 17 and continuing through line 27 as follows:

Full-length hKCNQ2 cDNAs were amplified from adult human brain cDNA using standard molecular biology techniques and the ~~following~~ primers ~~(~~CCCCGCTGAGCCTGAG (SEQ ID NO: 1), TGTAAAAGGTCACTGCCAGG (SEQ ID NO: 2)~~)~~ with the Expand Fidelity enzyme mixture (Boehringer Mannheim, Indianapolis, IN). The cDNA clone used in

biophysical and pharmacological studies was identical to the hKCNQ2 cDNA previously isolated from a fetal brain cDNA library by Singh, et al. (1998) except for a small deletion in the carboxy-terminal intracellular domain resulting in a 30 amino acid deletion of residues 417-446.

Please amend the paragraph beginning on page 19, line 1 and continuing through line 20 as follows:

PCR amplification of partial rKCNQ3 cDNA clones from rat brain and rat superior cervical ganglia (SCG) cDNA was performed. An initial sequence encompassing the entire open reading frame of the rKCNQ3 gene was determined through several rounds of 5' and 3' RACE PCR using initial anchor oligonucleotides complementary to the partial cDNA clone and SCG cDNA as a template for amplification. Once cDNAs were obtained that extended beyond both the 5' and 3' ends of the open reading frame, oligonucleotides complementary to non-coding regions at either end of the coding sequence were designed. Multiple full-length cDNA clones were amplified in independent PCR reactions from rat SCG cDNA using Expand Long Template PCR (Boehringer Mannheim, Indianapolis, IN) using several combinations of the following oligonucleotides: forward ~~(~~TTGACTCCCCATCCGACCT (SEQ ID NO: 3); GCCTTTGCCTTCTTTTGGG (SEQ ID NO: 4)~~)~~, reverse ~~(~~ACCGCGCACATGCATG (SEQ ID NO: 5), GTGACATGGGGAGGAAGAA (SEQ ID NO: 6)~~)~~. Four independent clones were sequenced in their entirety in both directions by automatic sequencing (GenBank accession number AF091247).

Please amend the paragraph beginning on page 22, line 1, and continuing through line 24 as follows:

Stock solution(s) of test compound(s) was (were) prepared immediately before use. An aliquot of the stock solution was diluted with an appropriate volume of bathing solution (as defined above) to attain a final working concentration(s) of test compound(s). Generally, the stock solution is about 0.1mM to about 100mM in DMSO and the aliquot is about 5 to about 100uL, but it is understood that the concentration and volumes are not limited ~~lkimited~~ to these ranges for one skilled in the art can readily determine the appropriate concentrations and volumes depending ~~dependening~~ on the activity of the compound in the assay and the sensitivity of detection. Recording of current through

KCNQ2+KCNQ3 channels as described above is performed prior to and at various times during the perfusion of the cell with a test compound solution. Current recordings can subsequently be made while perfusing the cell with a drug-free solution to determine reversibility of drug effects. Comparison of current amplitudes before test compound administration with those during administration indicate whether an agent affects channel activity. An example of an agent, XE991, that reversibly blocks hKCNQ2 expressed in HEK 293E cells is illustrated in Figure 5.

Please amend the paragraph beginning on page 23, line 22 and continuing through line 36 as follows:

Using the method of the invention, the selective blocking of the M-channel is demonstrated in HEK 293E cells stably expressing the hKCNQ2 potassium channel. Figure 6 illustrates that linopirdine induced a time- and concentration-dependent increase in fluorescence ~~flourescence~~ of HEK 293E cells stably expressing the hKCNQ2 potassium channel. These cells were loaded with the voltage-sensitive fluorescent dye, DiBAC, that distributes across cell membranes in a voltage-dependent manner. As cells depolarize (become more positive inside), more dye enters the cells and an increase in fluorescence occurs. Thus, these results indicate that under the conditions of the assay, linopirdine induced a time- and concentration-dependent depolarization which is believed to be mediated through a blockade of hKCNQ2.

Please amend the paragraph beginning on page 24, line 1, and continuing through line 19 as follows:

Using the method of the invention, the selective blocking of the M-channel is demonstrated in HEK 293E cells stably expressing the hKCNQ2 potassium channel. In Figure 7, the relative effects of several M-current modulators on the fluorescence ~~flourescence~~ of HEK 293E cells stably expressing the hKCNQ2 potassium channel is shown. These cells were loaded with the voltage-sensitive fluorescent dye, DiBAC, that distributes across cell membranes in a voltage-dependent manner. As cells depolarize, more dye enters the cells and an increase in fluorescence occurs. Conversely, as cells hyperpolarize (become more negative inside), more dye leaves the cells and a decrease in fluorescence occurs. Thus, these results indicate that, at 3 μM, XE991 and XR543 induced more membrane depolarization than linopirdine. In addition, at

3 μM, X7315 exerted no effect whereas, at 100 μM, X7315 induced an apparent membrane hyperpolarization. These results suggest that this fluorescence assay can be utilized to detect both blockers and openers of the hKCNQ2 channel.

Please amend the paragraph beginning on page 26, line 34, and continuing through page 35, line 3 as follows:

The compounds identified using the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches wall known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent ~~intermittant~~ throughout the dosage regimen.